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A prospective study to assess the clinicopathological and prognostic value of BRAF V600E mutation in metastatic colorectal cancer

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Abstract

Introduction: BRAF is a protein kinase downstream of RAS in the RAS-RAF-MEK-ERK kinase pathway, the prevalence of BRAF mutation might be underestimated, while it is considered a major negative prognostic marker and is associated with resistance to standard chemotherapeutic regimens in metastatic colorectal cancer (mCRC) patients, justifying a personalized therapeutic approach in BRAF-mutated mCRC patients. Given this poor outcome in patients with BRAF-mutated mCRC, optimizing therapy is an important goal.
Objectives: This study aimed to study BRAF mutation in patients with metastatic CRC and its association with clinicopathological factors and survival outcomes.
Patients and Methods: This prospective study included 55 patients with histologically proven CRC with metastatic disease, either radiologically or pathologically proven. BRAFV600E mutation analysis was performed using the polymerase chain reaction (PCR) and reverse hybridization method on formalin-fixed and paraffin-embedded extracted DNA samples.
Results: In our study, 55 patients were enrolled. The mean age was 49.5 years with male predominance. Among patients enrolled in the study, 54 were evaluated for survival over one year. The median progression-free survival (PFS) was 11.167 months, and the median overall survival (OS) was 34.5 months. BRAF V600E mutation was detected in 9.1% of patients and all of them presented with synchronous metastasis, with statistical significance (P = 0.0339). No significant difference was observed in clinicopathological factors, PFS, or OS between BRAF-mutant and wild-type patients. Only 48 patients were evaluated for their response to first-line therapy; it was found that most patients who did not receive targeted therapy had progressive disease with a statistical significance of P = 0.0450. A median PFS of 19.2 months was also noted with statistical significance (P = 0.0121).
Conclusion: The BRAFV600E mutation is associated with more aggressive features, but no association with PFS or OS was found. Chemotherapy with the addition of targeted therapy has an impact on PFS. Further investigations are therefore warranted and the inclusion of BRAF-mutated mCRC patients in clinical trials needs to be encouraged.

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Introduction

Colorectal cancer (CRC) is the third most often diagnosed malignancy and the second major cause of cancer mortality. Colorectal cancer incidence rates in developed countries are generally four times greater than in developing countries (1).

Despite substantial breakthroughs in CRC therapy over the last 15 years, the disease continues to be the most significant cause of cancer-related mortality globally (1).

At the time of diagnosis, about 20% of CRC patients have metastases, and more than 50% develop metastatic disease during the entire duration of their disease (2).

Over the last decade, molecular testing in patients with metastatic CRC (mCRC) has become routine practice, and knowledge of RAS, BRAF, and microsatellite instability status is now necessary to provide patients with optimal therapy. This has resulted in a better clinical outcome for individuals with mCRC (3).

Since BRAF (B-Raf proto-oncogene, serine/threonine kinase) is a protein kinase downstream of RAS in the RAS-RAF-MEK-ERK kinase pathway, its incidence may be underestimated because individuals with

Key point

BRAF is an important protein kinase downstream of RAS in the RAS-RAF-MEK-ERK kinase pathway; however, the prevalence of BRAF mutation may be underestimated, despite the fact that it is a major negative prognostic marker and is associated with resistance to standard chemotherapeutic regimens in mCRC patients, justifying a personalised therapeutic approach in BRAF-mutated mCRC patients.

these mutations are usually ruled out for clinical trials due to their poor performance status and age. Indeed, the incidence of BRAF mutations in CRC patients was recently shown to be as high as 21% in a Norwegian registry (4).

The largest proportion of BRAF mutations in mCRC (>95%) occur in codon 600, involving a T1799A transversion in exon 15, resulting in a valine amino acid substitution for a glutamic acid (V600E mutation). BRAF mutations other than V600E are identified in 2% of mCRC patients and indicate a clinically separate subtype with a better prognosis (5).

The BRAFV600E mutation is a prominent unfavourable prognostic factor in mCRC patients and is frequently associated with resistance to conventional chemotherapy regimens, suggesting a personalised treatment strategy in BRAF-mutant mCRC patients.

In a recent retrospective analysis of 2084 mCRC patients, patients with non-V600E BRAF mutations had an overall survival (OS) of 39.4 months, yet patients with V600E BRAF mutations had an OS of just 21 months (6).

Furthermore, a meta-analysis of 11 321 patients revealed that the mortality risk for those with BRAF-mt (BRAF-mutated) disease was more than double that of those with BRAF-wild-type disease (7).

In randomised phase 3 clinical trials, patients with RAS wild-type mCRC (8) had a median OS of more than 30 months; for mCRC patients who were not chosen based on RAS status, the median OS was between 9 and 25 months. These approaches combine a targeted drug with doublet or triplet chemotherapy (9).

A recent meta-analysis of patients with KRAS wt mCRC discovered that those with BRAF-mt/KRAS wt disease had substantially worse survival, with a median OS of 10.8 months (10).

Given this poor outcome, we will focus our efforts in this study on BRAF mutations in mCRC, specifically the V600E mutation, as well as their clinical significance, molecular and clinicopathological factors, treatment received, and survival outcomes, aiming to develop novel treatment options to improve patient outcomes.

Objectives

This study aimed to study BRAF mutation in patients with metastatic CRC and its association with clinicopathological factors and survival outcomes.

Patients and Methods

Study design

This is a prospective study including patients diagnosed with mCRC, presented at the Department of Clinical Oncology, Ain Shams University Hospitals from March 2020 to March 2021 with follow-up of at least 12 months.

Patients

Eligible patients for this study, aged ≥18 years, Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2, with adequate hematologic, liver, and renal functions, had pathologically confirmed CRC with metastatic disease, either radiologically or pathologically proven. Whereas patients with a second primary malignancy, inadequate or insufficient tissue samples were excluded.

Response, survival assessment

Disease response was assessed every 3 to 4 months and patients were followed for at least 12 months to assess survival. Response assessment is based on Modified Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 (11). Progression-free survival (PFS) was calculated from the date of diagnosis as a metastatic patient to objectively documented radiographic tumor progression or death, while OS was calculated from the date of diagnosis to the date of last follow-up, lost to follow-up, or death.

Tissue collection

This study included 55 specimens of formalin-fixed and paraffin-embedded tumor sections. Cases were retrieved from the Clinical Oncology Department of Ain Shams University Hospitals, Cairo, Egypt. Full history was taken from the patients and all clinical and pathological data needed was documented.

All cases included in this study were selected to have sufficient representative tissues for evaluation.

Methodology

The procedure includes three steps: (a) DNA isolation, (b) polymerase chain reaction (PCR) amplification using biotinylated primers, (c) hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

Assay procedure

DNA isolation: Appropriate DNA extraction methods were applied depending on the type of specimen. DNA concentration was adjusted to 1–10 ng/μL for use in the BRAF 600/601 Strip Assay®.

In vitro amplification (PCR): All PCR reagents and DNA templates were refrigerated throughout. All steps are performed until the start of the thermal cycling programme on ice (0–4 °C).

Hybridization (45 °C; shaking water bath): The water level of the water bath was adjusted to approximately ½ of the height of the typing tray. The water bath was heated to exactly 45 °C (± 0.5 °C). accordingly, the water temperature was checked with a calibrated thermometer. The hybridization buffer and solution A were both prewarmed to 45 °C. Test strips, DNAT, conjugate solution, the wash of solution B, and colour developer were allowed to reach room temperature, and typing tray(s) were prepared. Then

one test strip for each sample was removed using clean tweezers. The test strips outside of the marker lines were labelled with a pencil.

Interpretation of results

The genotype of a sample was determined using the enclosed Collector TM sheet. The processed test strip was placed into one of the designated fields, aligned to the schematic drawing using the red marker line (top) and the green marker line (bottom), and then fixed with adhesive tape. A positive reaction in the uppermost control line indicates the correct function of the conjugate solution and colour developer. This line should always be positive. A positive reaction of the PCR positive control indicates the presence and adequate quality of PCR components and DNA template for BRAF analysis. If the PCR-positive control showed negative results on a DNA template, the analysis was repeated, starting with DNA preparation. A negative reaction to the PCR negative control indicates complete suppression of wild-type BRAF amplification. If the PCR negative control is positive (e.g., due to excess DNA template used for PCR), the sensitivity of the assay may be impaired.

Statistical analysis

Baseline clinic-epidemiological factors, laboratory factors, and molecular ones are expressed as absolute values, mean ± standard deviation, and median (range) when appropriate. A chi-square test was conducted to assess the association between these parameters. Results will be shown as *P* values. Statistical significance was defined as $P \leq 0.05$. The value of $P < 0.01$ was highly significant whereas the $P > 0.05$ was non-significant.

Results

This prospective study was conducted at the department of clinical oncology and nuclear medicine, Ain Shams university. The study included patients who were radiologically or pathologically proven to have CRC with metastatic disease to assess the clinicopathological and prognostic value of the BRAF V600E mutation for a duration of at least one year.

Descriptive analysis

Fifty-five patients were included in our study. The mean age of the patients studied was 49.5 years, while the median was 50 years (range: 21-77 years). Table 1 shows patients' characteristics.

Table 1. Demographic and clinical characteristics of the metastatic colorectal cancer patients (n=55)

	Number	Percent
Gender		
Female	24	43.6
Male	31	56.4
Residence		
Rural	17	30.9
Urban	38	69.1
Comorbidity		
Hypertension	14	25.5
Diabetes mellitus	9	16.4
Hepatitis C virus	4	7.3
Smoking		
No	48	87.3
Yes	7	12.7
Complaint		
Bleeding per rectum	23	41.8
Abdominal pain	14	25.5
Change bowl habits	14	25.5
Obstruction	13	23.6
Weight loss	5	9.1
Performance status		
I	42	76.4
II	13	23.6
Positive family history		
Colorectal cancer	14	25.5
Others	7	12.7

Pathological characteristics

The most common tumour site was the left colon (approximately two-thirds of cases), with rectal cancer accounting for 41.8%, followed by the sigmoid colon and the right colon. Approximately 54.5% of patients had synchronous metastasis. In 50.9% of patients, liver metastases were the most common site of metastasis, followed by nodal metastases (non-regional), lung metastases, and peritoneum. As shown in Table 2, the BRAF V600E mutation was detected in 9.1% of patients, while the RAS mutation was detected in 12.7% of them.

Treatment

Fifty-four patients were treated with first-line chemotherapy, while one patient only had metastasectomy and follow-up. Approximately 66.7% of cases were treated with oxaliplatin-based chemotherapy, while 22.2% were treated with irinotecan doublet chemotherapy. Only 15 patients received target therapy, 53.3% of whom received bevacizumab (Table 3). The median duration of treatment was 7 months (range: 1.0-20 months).

Correlation between BRAF mutation and clinicopathological factors

No statistical significance was found when correlating the BRAF mutation with clinical and pathological factors. However, regarding the timing of metastasis, it was found that all patients with BRAF mutation had synchronous metastasis with a statistically significant *P* value of 0.0339

Table 2. Pathological characteristics of metastatic colorectal cancer patients

	Number	Percent
Side		
Left	39	70.9
Right	15	27.3
Right and right	1	1.8
Pathology		
Adenocarcinoma (NOS)	41	74.5
Mucinous	9	16.4
Signet ring	5	9.1
Metastatic sites		
Liver	28	50.9
Lung	18	32.7
Bone	4	7.3
Nodal (non-regional)	23	41.8
Peritoneum	13	23.6
Timing of metastasis		
Metachronous	25	45.5
Synchronous	30	54.5
BRAF mutation		
Mutant	5	9.1
Wild	50	90.9
RAS mutation		
Mutant	7	12.7
Wild	48	87.3

Table 3. Lines of treatment received (N=55)

	Number	Percent
First-line target therapy		
Yes	15	27.2
Type		
Bevacizumab	8	53.3
Cetuximab	4	27.7
Panitumumab	3	20
First line chemotherapy		
Irinotecan duplet	12	22.2
Oxaliplatin duplet	36	66.7
Single agent	6	11.1
Second line chemotherapy		
Irinotecan duplet	19	70.4
Oxaliplatin	4	14.8
Single-agent	3	11.1
Triplet	1	3.7
Second-line target therapy		
Bevacizumab	6	50
Cetuximab	3	25
Panitumumab	3	25
Third line chemotherapy		
Irinotecan duplet	2	20
Oxaliplatin duplet	4	40
Single-agent	4	40
Third line target therapy		
Bevacizumab	4	50
Cetuximab	1	12.5
Regorafenib	1	12.5
Panitumumab	2	25

(Table 4).

Correlation between BRAF and target therapy with first-line response

Only 48 patients were evaluated for response after first-line therapy. There was no statistical significance when correlating BRAF status with first-line response ($P=0.3727$). However, when correlating the first-line response with target therapy, it was found that most patients (39%) statistically showed progressive disease who did not receive targeted therapy ($P=0.0450$), as shown in Table 5.

Survival

Among the 55 patients who were enrolled in the study, 54 patients were assessed for survival with follow-up for at least 1 year. At the end of the follow-up period, 39 patients were alive and 16 dead. Median disease-free survival was 13 months, median PFS was 11.167 months, and median OS was 34.5 months (Figures 1 and 2).

Correlation between BRAF mutation and PFS

The median PFS of patients with BRAF mutation was 8.067 months, while it was 11.167 months for patients with wild BRAF, with no statistically significant difference ($P=0.789$; Figure 3).

Correlation between BRAF mutation and overall survival

There was no significant difference in median OS between patients with mutated BRAF compared to those with wild type (39.6 versus 34.5 months; $P=0.343$; Figure 4).

Correlation between variables and PFS

None of the demographic characteristics, including age, gender, and all other characteristics, had a significant impact on PFS. Tumour site, pathology, time of metastasis, and site of metastasis also had no statistical significance on PFS. As for first-line treatment. In terms of first-line therapy, there was no statistically significant difference in PFS between chemotherapy types received. Patients who received targeted therapy as first-line had a median PFS of 19.2 months, with statistical significance ($P=0.0121$; Figure 5), as shown in Table 6. Furthermore, when correlating response after first-line treatment with PFS, patients with disease progression after first-line treatment had a much lower median PFS than patients with partial response or stationary disease. (8 months versus 14 and 15 months) with statistical significance ($P=0.0132$; Figure 6).

Correlation between variables and overall survival

Age, gender, family history of CRC, side of tumour, and pathologic factors all had no influence on OS. Regarding

Table 4. Correlation between BRAF mutation and clinicopathological variables

Category	Mutant	Wild	*P* value
Age (y)			
<40	1 (1.8%)	13 (23.6%)	0.7711
≥40	4 (7.3%)	37 (67.3%)	
Gender			
Male	3 (5.5%)	28 (50.9%)	0.8647
Female	2 (3.6%)	22 (40%)	
ECOG PS			
1	4 (7.3%)	38 (69.1%)	0.7168
2	1 (1.8%)	12 (21.8%)	
Residence			
Rural	2 (3.6%)	15 (27.3%)	0.6476
Urban	3 (5.5%)	35 (63.6%)	
FH			
No	4 (7.3%)	37 (67.3%)	0.7711
Yes	1 (1.8%)	13 (23.6%)	
FH (colorectal cancer)			
No	4 (7.3%)	44 (80%)	0.6121
Yes	1 (1.8%)	6 (10.9%)	
Side			
Left	2 (3.65%)	37 (67.3%)	0.1138
Right	3 (5.5%)	13 (23.6%)	
Pathology			
Adenocarcinoma (NOS)	4 (7.3%)	37 (67.3%)	
Mucinous	1 (1.8%)	8 (14.5%)	0.7534
Signet ring	0 (0%)	5 (9.1%)	
Site of metastasis			
Liver			
Yes	3 (5.5%)	25 (45.5%)	0.6726
No	2 (3.6%)	25 (45.5%)	
Lung			
Yes	3 (5.5%)	15 (27.3%)	0.1768
No	2 (3.6%)	35 (63.6%)	
Peritoneum			
Yes	1 (1.8%)	12 (21.8%)	0.8423
No	4 (7.3%)	38 (69.1%)	
Lymph nodes			
Yes	3 (5.5%)	20 (36.4%)	0.3917
No	2 (3.6%)	30 (54.5%)	
Metastatic timing			
Metachronous	0 (0%)	25 (45.5%)	0.0339
Synchronous	5 (9.1%)	25 (45.5%)	
First-line target therapy			
Yes	2 (3.7%)	13 (24.1%)	0.5257
No	3 (5.6%)	36 (66.7%)	
First line chemotherapy			
Irinotecan duplet	0	12 (22%)	
Oxaliplatin duplet	4 (7.4%)	32 (59.3%)	0.4141
Single agent	1 (1.9%)	5 (9.3%)	

PS: Performance status; ECOG: Eastern Cooperative Oncology Group; FH, Family history.

Table 5. The relationship between variables and 1st line response (n=48)

Category	Progressive disease	Partial response	Stationary disease	*P* value
BRAF				
Mutant	3 (6.2%)	1 (2.1%)	0 (0%)	0.3727
Wild	18 (37.5%)	17 (35.4%)	9 (18.8%)	
Target therapy				
No	19 (39.6%)	10 (20.8%)	6 (12.5%)	0.0450
Yes	2 (4.2%)	8 (16.7%)	3 (6.2%)	

first-line treatment, patients who received oxaliplatin duplet had a median OS of 37.56 months, higher than those who received irinotecan duplet with a median OS of 25.4 months and those who received single line of treatment with an OS of 14 .2 months, but without statistical significance ($P=0.1659$; Table 7). Patients who did not receive target therapy with first-line treatment had a much lower median OS than those who did with a median OS of 25.4 and 38.56 months, respectively, but without statistical significance ($P=0.2714$; Figure 7).

Discussion

BRAF is an important downstream effector of RAS in the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signal transduction pathway, which mainly affects cell proliferation, differentiation, and apoptosis. BRAF is therefore considered an oncogenic driver in colorectal tumors. BRAFV600E mutation is an important negative prognostic marker and is associated with resistance to standard chemotherapeutic regimens in mCRC patients, warranting a personalized therapeutic approach in mCRC patients with BRAF mutation (6).

This study was conducted to determine the clinicopathologic and prognostic role of the BRAF V600E mutation in patients with metastatic CRC.

BRAF mutations were identified in approximately 5%–10% of CRC patients in different patient cohorts (14). A report that included 2530 patients with mCRC enrolled in three randomized trials (COIN, FOCUS and PICCOLO), showed the prevalence of BRAF mutations was 9.1%

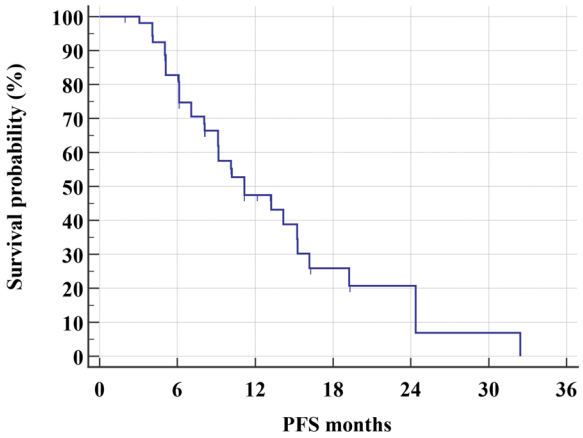

Figure 1. Progression-free survival (PFS): All patients (n=54).

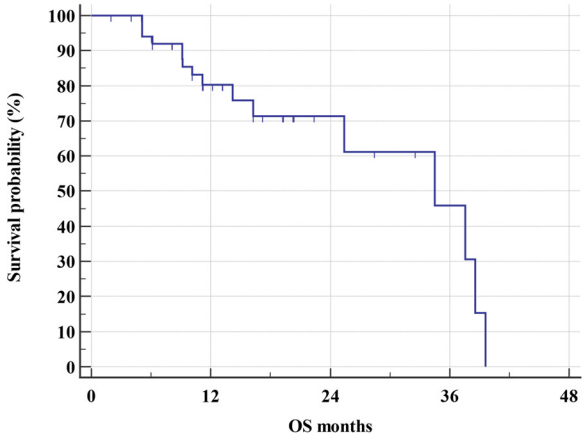

Figure 2. Overall survival (OS): All patients.

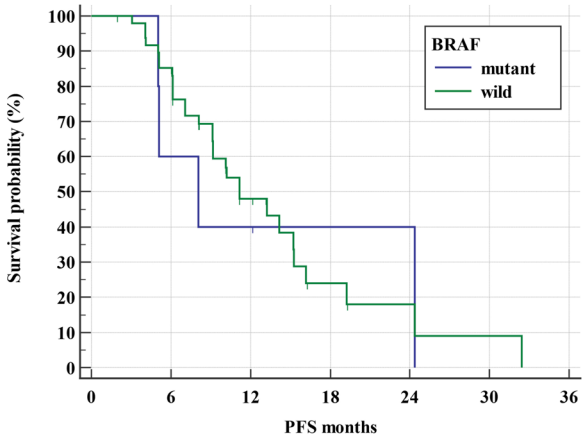

Figure 3. Correlation between BRAF mutation and progression-free survival (PFS). 1-year PFS rate: Mutant BRAF = 40%; Wild BRAF = 48%.

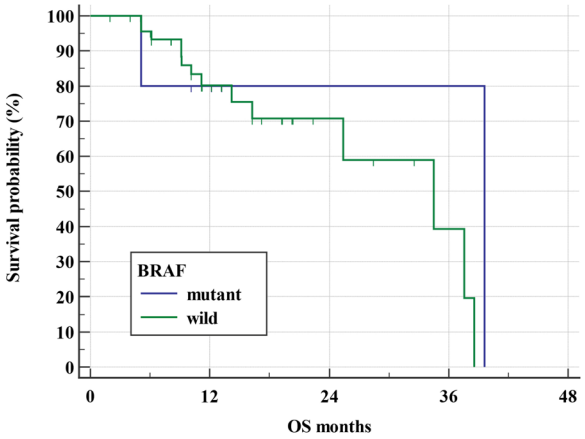

Figure 4. Correlation between BRAF mutation and overall survival. 1-year OS rate: Mutant BRAF = 80%; Wild BRAF = 80.2%.

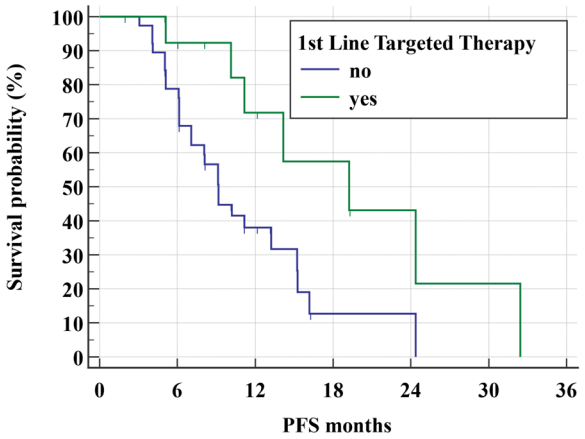

Figure 5. Correlation between patients who received targeted therapy as first-line and progression-free survival (PFS).

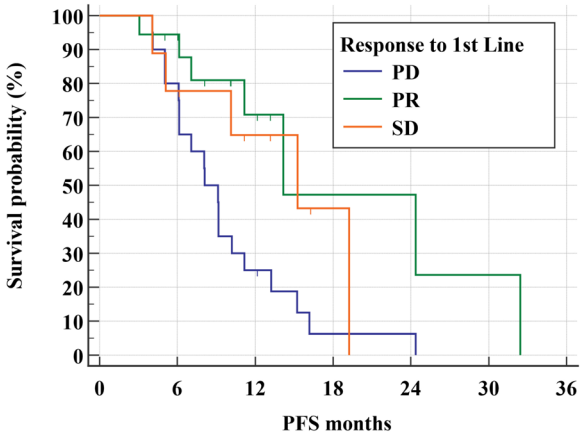

Figure 6. Correlation between responses after first-line treatment with progression-free survival (PFS).

(12) Prospective clinical data and molecular analyses performed on patients with metastatic CRC, overall, 11% of patients had BRAF mutation-bearing tumours (15). All of this is consistent with the results of our study, which showed that approximately 9.1% of all included patients had a BRAF mutation.

In our study, it was found that the incidence of BRAF V600E mutation was higher in males, and this is inconsistent with most studies that showed that BRAF V600E mutation is strongly associated with female gender, however, in a retrospective case-series study, the male to female ratio for V600E BRAF was found to be 1.4:1. Other studies from Eastern populations showed male dominance or no dominance (16).

As with most cancers, the risk of CRC increases with age. In our study, it was found that the average age of the patients is 50 years. It was also found that the BRAF mutation was more common in patients aged 40 years or more, but without statistical significance.

The definition of age to be considered young is controversial. In an analysis of 6,425 patients, O'Connell et al (17) found that 37 manuscripts considered people under the age of 40 to be young. In the literature, most publications refer to the CRC incidence in patients under the age of 4018. On the other hand, when studying the correlation between age and BRAF using a cut-off of 60 years old, the association between BRAFV600E mutation

Table 6. Correlation between variables and progression-free survival

	N	Median PFS (months)	*P* value
First line			
Irinotecan duplet	12	13.233	0.708
Oxaliplatin duplet	35	10.200	
Single-agent	6	9.133	
First line target			
Yes	15	19.233	0.0121
No	38	9.167	
First line response			
Progressive disease		8.100	0.0132
Partial response		14.167	
Stationary disease	9	15.267	
Gender			
Male	30	10.200	0.8702
Female	24	11.167	
Age groups (y)			
≤40	14	28.57	0.5421
>40	40	40.0	
ECOG			
1	41	11.167	0.5912
2	13	11.167	
FH of 1-year OS rate Mutant BRAF = 80%; Wild BRAF = 80.2%			0.1831
No	48	11.167	
Yes	6	5.100	
Side			
Left	38	13.233	0.6361
Right	16	8.100	
Pathology			
Adenocarcinoma	41	11.167	0.6226
Mucinous	8	10.200	
Signet ring	5	7.067	
Mets timing			
Metachronous	24	13.233	0.4319
Synchronous	30	11.167	

ECOG: Eastern Cooperative Oncology Group; FH, Family history; PFS, Progression-free survival; OS: overall survival.

Table 7. Correlation between variables and overall survival

	N	Median OS (months)	*P* value
First line			
Irinotecan duplet	12	25.400	0.1659
Oxaliplatin duplet	35	37.567	
Single agent	6	14.200	
First line target			
Yes	15	38.56	0.2714
No	38	25.4	
First line response			
Progressive disease	20	25.400	0.9638
Partial response	18	34.500	
Stationary disease	9	-	
Gender			
Male	30	37.567	0.5471
Female	24	25.400	
Age groups (y)			
≤40	14	25.400	0.6928
>40	40	37.567	
ECOG			
1	41	37.567	0.0448
2	13	14.200	
FH of colorectal cancer			
No	48	37.567	0.7473
Yes	6	25.400	
Side			
Left	38	34500	0.6544
Right	16	38.567	
Pathology			
Adenocarcinoma (NOS)	41	34.500	0.7916
Mucinous	8	-	
Signet ring	5	-	
Metastasis timing			
Metachronous	24	37.567	0.4602
Synchronous	30	34.500	

ECOG: Eastern Cooperative Oncology Group; FH, Family history; OS: overall survival.

and age did not reach statistical significance.

Most cases of CRC occur in people without a family history of CRC or a predisposing condition and this is consistent with our research confirming that 75% of our patients had no identified family history of CRC.

Based on a 6-year institutional retrospective follow-up study, it was found that a large percentage of patients (65.7%) were diagnosed at late stages. 60.4% of patients diagnosed as stage IV and approximately 488 (78.6%) were of the adenocarcinoma type, consistent with our study results which showed that approximately 56.6% were presented with stage IV from the start and about 74.5% were presented with adenocarcinoma type (18).

In BRAF-mt mCRCs, the proximal colon was found to be the preferred location, suggesting that the genomic alterations in the proximal and distal colonic mucosa produce different CRC phenotypes (19). A total of 45 studies examined the relationship between BRAFV600E mutation and tumour site. The final results showed that the BRAFV600E mutation was associated with the location of the tumor in the proximal colon or the right colon (20). Similarly, in our study, it was found that approximately 60% of patients with BRAF mutation presented with a right-sided tumor, but without statistical significance, and this can be attributed to the small sample size in our study.

In our study, it was found that more patients had synchronous metastases rather than metachronous metastases, consistent with most studies, the median survival of the synchronous and metachronous patients was 34.5 and 37.56 months, respectively, but due to the

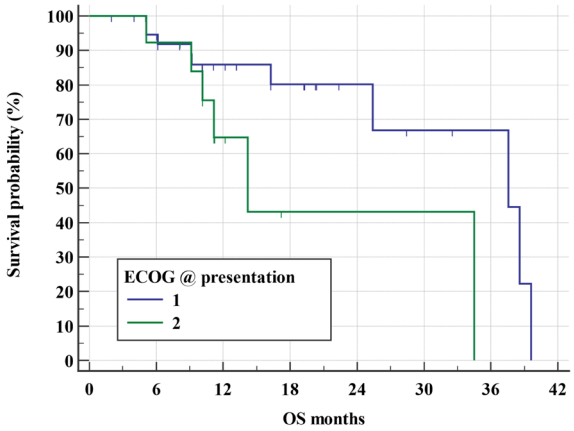

Figure 7. Correlation between ECOG at presentation and overall survival.

small sample size in our study, the difference was not statistically significant.

In a retrospective study performed on 1,672 patients, the incidence of synchronous and metachronous metastases was 16% and 7.7%, respectively. Patients with synchronous and metachronous metastases had a median survival of 10 and 43 months, respectively (21).

There was little data on the molecular difference and its impact on synchronous and metachronous metastasis in CRC patients. Kim et al (22) identified mutations in major pathway genes, including KRAS, BRAF, PIK3CA, TP53, APC, and NRAS, and similar mutational profiles were observed in patients with synchronous and metachronous metastasis. Fujiyoshi et al (23) reported that there were high concordance rates of KRAS and BRAF mutations between primary CRC tumor tissue and metastatic tissue; However, the high concordance rates of these genes did not differ significantly between patients with synchronous and metachronous metastases. It seems that the genetic changes between patients with synchronous and metachronous metastases were not significantly different.

On correlating PFS and OS with the clinicopathological parameters; such as age, gender, tumor location, and mucinous histology, no statistically significant difference could be detected and this was consistent with a prospective study performed on 504 patients with metastatic CRC and the result showed that there was no significant impact when these parameters were correlated with PFS and OS. The negative impact of the BRAFV600E mutation has been reported in patients with advanced CRC, in a pooled analysis involving more than 3000 patients from the CAIRO, CAIRO 2, COIN, and FOCUS trials, patients with the BRAFV600E mutation showed both worse PFS and OS (24).

Concerning PFS when correlated with BRAF mutation in our study, it was found that the median PFS of patients with BRAF mutation was lower (8.067 months) than that of those with wild BRAF (11.167 months), but with no statistically significant difference ($P=0.789$). Regarding OS, patients with BRAF mutation had a median OS of 39.6 months, while in wild-type patients it was 34.5 months and there was no statistical significance shown.

Treatment response is limited in BRAF-mt CRC patients; a retrospective study by Morris V et al reported no differences in PFS whether oxaliplatin- or irinotecan-based chemotherapy was administered in first-line therapy (6.4 versus 5.4 months) (25).

Another retrospective analysis of the mutation status from the FOCUS study also showed no significant differences in the treatment outcomes between patients with different KRAS or BRAF status (26), as in another analysis performed on 2530 patients evaluated in three large randomized trials (FOCUS, COIN, and PICCOLO), no significant differences in adjusted PFS between BRAF mutation-positive and wild-type patients receiving chemotherapy (27).

All of these were consistent with our study, which showed that there was no statistical significance when correlating PFS with the lines of treatment received, whether it was oxaliplatin- or irinotecan-based chemotherapy. The correlation of the BRAF status with the received treatment lines also showed no statistical significance.

Studies comparing first-line monoclonal antibodies in advanced colon cancer (CRC) have yielded conflicting results. Recent studies have confirmed the efficacy of bevacizumab in combination with other chemotherapy drugs in mCRC and show its increasing use in clinical practice. In our study, targeted therapy, which was received in the first-line setting with chemotherapy drugs, was found to have statistical significance for PFS and response while not statistically affecting OS in our entire enrolled population.

However, it was found that patients who received target therapy plus chemotherapy had a median OS of approximately 38.5 months, compared with 25.4 months in those who did not receive any, with a difference of approximately 13 months in OS between the two groups, keeping with most of the studies that have been done that showed the statistical significance of adding target therapy to the chemotherapy drugs on survival in patients with metastatic CRC.

In February 2004, the United States Food and Drug Administration (FDA) approved bevacizumab for the first-line treatment of patients with metastatic colon and rectal cancer based on a study, where 833 patients were randomized to the IFL regimen alone or with bevacizumab. In the bevacizumab-treated group, OS was significantly longer, as was PFS and response rate (28).

oxaliplatin-based chemotherapy in combination with bevacizumab was also evaluated in the NO16966 trial (29) the median PFS was significantly improved in the bevacizumab-containing arm compared to the placebo arm.

Regarding the addition of anti-EGFR agents, the efficacy of cetuximab in combination with chemotherapy in the

first-line treatment of mCRC was evaluated in two pivotal clinical studies: the phase III CRYSTAL study and the phase II OPUS study (30,31).

In the CRYSTAL study, the benefit of adding cetuximab to FOLFIRI was demonstrated by a significant improvement in RR, PFS, and OS compared to FOLFIRI alone. In the OPUS study, in the KRAS wild-type population, patients treated with cetuximab in combination with FOLFOX-4 showed higher RR and better PFS but it did not show OS benefit. In contrast, the phase III COIN study, in which patients with mCRC randomized to an oxaliplatin-based regimen with or without cetuximab, showed no benefit of adding cetuximab to chemotherapy in terms of PFS and OS compared to chemotherapy alone, even in the KRAS wild-type population (32,33).

Regarding BRAF-mutant mCRC, there is limited evidence that the addition of anti-EGFR therapy to chemotherapy results in a clinically significant benefit (32,34). A systematic review and meta-analysis of randomized controlled trials examined the effect of BRAF mutations on treatment benefit from anti-EGFR therapy for mCRC. The authors concluded that there is insufficient evidence that anti-EGFR therapy in BRAF-mutant tumors (35) affects neither PFS nor OS. In the pooled analysis data from the randomized CRYSTAL and OPUS studies, there was an improvement in objective response rate, PFS and OS in the subgroup of BRAF-mt-mCRC patients but these differences were not statistically significant (34).

The role of bevacizumab in BRAF-mt mCRC patients has not yet been clinically established. In the first study to report the high efficacy of bevacizumab (30), the median OS was 16 months in patients with BRAF-mutated tumors compared to 8 months in patients who received chemotherapy alone. However, the number of patients included in this post-hoc analysis was very small (10 patients) (36). Although no randomized data evaluating the impact of adding bevacizumab to standard chemotherapy of Patients with BRAF-mt mCRC, the addition of bevacizumab to first-line IFL treatment or capecitabine has a numerical improvement in survival outcomes in patients with BRAF-mt in post hoc analysis of the AVF2107g (36) and AGITG MAX (37) studies.

Considering the data and results of several studies to date, our study showed almost the same end result that there was no statistical significance for adding a target therapy, either anti-EGFR agents or bevacizumab, to chemotherapy drugs in patients with BRAF mutation.

Conclusion

Although BRAF mutations are uncommon in mCRC, they have a significant unfavourable effect upon clinical presentation, histology, molecular characteristics, patient prognosis, and treatment options. However, unlike other BRAF mutated cancers, this is strongly associated with and dependent on the genetic and epigenetic background that may develop during disease and therapy. In reality, the incidence of BRAF mutations in early stage CRC is likely underestimated, and its predictive usefulness in this situation remains unknown, as evidenced by the poor and conflicting results published until now.

Given the poor outcome for people who have BRAF-mt mCRC, optimising treatment is a vital priority.

Limitations of the study

Our study is considered the first to report the impact of BRAFV600E mutation in metastatic CRC among the Egyptian population. Our study had several limitations as our study sample is small which may not be representative of the whole population.

There was no statistically significant difference in PFS or OS between BRAF-mutant and wild-type patients. Further studies with a larger sample size are warranted to determine the prognostic and predictive value of BRAFV600E mutation in metastatic CRC patients.

Authors' contribution

Conceptualization: All authors.
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Visualization: Mona Taher Aboulfadl.
Writing–original draft: Mona Taher Aboulfadl.
Writing–review & editing: All authors.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the faculty of medicine, Ain Shams university ethical committee with approval number FWA000017585. The study was based on data collection and immunohistochemical analysis of positively charged slides prepared from paraffin blocks so informed consent was not applicable to our study. This study was extracted from the MD thesis of Mona Aboulfadl registered at the faculty of medicine, Ain Shams university. Additionally, ethical issues (including plagiarism, data fabrication, and double publication) have been completely observed by the authors.

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